CHOLESTEROL METABOLISM.

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THE one certain conclusion that can be drawn from the very large amount of work done on this subject during the past fifty years is that cholesterol is an essential constituent of every cell of the body, just as are protein, fat and carbohydrate. It is present in all body fluids; even urine contains traces, much augmented in albuminuria. Chemically, cholesterol is an unsaturated secondary alcohol with one doubly-linked pair of carbon atoms. It exists in the body in the free or alcoholic condition, and also in the form of its esters with the higher fatty acids—oleic, palmitic and stearic acids. These two forms are not equally distributed. Brain and the red blood corpuscles, for example, contain it almost entirely in the free state. Two samples of moist adult human brain contained respectively 1·95 and 1·91 per cent. of cholesterol, but no cholesteryl esters; the brain of a three-months' old child 0·69 per cent., all free; average for brain of three still-born children, 0·56 free and 0·006 per cent. ester, and a fœtus (five to six months) 0·44 free and 0·009 ester.

Analyses of five samples of moist human red blood corpuscles gave an average of 0·074 per cent. free cholesterol, with individual variations of 0·062-0·081, and 0·003 cholesterol as esters.

In human bile the percentage of cholesterol is very variable, but mainly in the free condition, and probably in close association with bile acids.

Other tissues contain both free cholesterol and cholesteryl esters. For example:—

Grammes of Cholesterol per 100 *Grammes of Dried Normal Organ* (Fex.)

Organ.	Total Cholesterol.		Free Cholesterol.		Ester Cholesterol.	
	Average.	Limits of Variation.	Average.	Limits of Variation.	Average.	Limits of Variation.
Liver . . .	1·283	0·96- 1·55	1·024	0·71-1·31	0·258	0·17- 0·32
Kidney . . .	1·661	1·37- 2·01	1·540	1·14-1·91	0·109	0·06- 0·15
Supra-renal (whole) .	16·187	10·71-19·98	2·611	1·40-3·00	13·800	7·93-17·35

https://doi.org/10.1017/S0022215100037427 Published online by Cambridge University Press

In the above table the "cholesterol" is, strictly speaking, the sterol precipitable by digitonin in the form of a digitonide insoluble in alcohol and ether. This sterol is in fact mainly cholesterol, but, as Schönheimer has recently shown, may contain small quantities—2 to 4 per cent. of the whole—of a saturated sterol, viz., bihydrocholesterol, which is also precipitable by digitonin.

In studying the metabolism of the human subject, attention has been directed mainly to the blood and excreta, since other organs can be dealt with only post-mortem.

In the red cells, where the cholesterol is mainly in the free condition, the percentage appears to be fairly constant both in health and disease. On the other hand, the cholesterol of the plasma changes markedly during both the ordinary metabolic processes and in disease. This variation takes place, in healthy metabolism, mainly in the ester portion. For this reason it is *essential* that blood cholesterol estimations should be made on the plasma, or at any rate on the serum, so that changes in the cholesterol content should not be masked by the rather high constant mass of corpuscular cholesterol; and further, that the interpretation of results should not be complicated by the presence of anæmia.

It is very important that both free and ester cholesterol should be estimated, otherwise we get only a part of the story, and often the less interesting part.

During the past twenty years a vast amount of work has been published on the amount of cholesterol in the blood in disease. Unfortunately, in earlier years most writers dealt only with the total cholesterol, and even at the present day we find too many workers doing the same, as though they regarded the esterification as merely fortuitous. This unfortunate position may be explained by the historical development of the subject. The ether extract of tissue contains fatty acids, fats, cholesterol, cholesterol esters, etc., a mixture often lumped under the misleading and undefinable term—lipoids. In early days the separation of these constituents presented many difficulties, and their association led to the idea that cholesterol is intimately connected with fat metabolism—a view in support of which there is little real evidence. This idea has persisted, however, and has been largely insisted on by the French school of observers.

Space does not, however, permit of a discussion of the various

https://doi.org/10.1017/S0022215100037427 Published online by Cambridge University Press

theories that have been propounded on sterol metabolism, and indeed at the present time we have not got the data on which to base a really sound theory, or even effectively to criticise hypotheses propounded from time to time. I propose, therefore, simply to draw attention to certain biochemical facts, accumulated during the past decade, which must be taken into account in any proper interpretation of results.

(1) It is important to realise at the outset, in dealing with hypercholesterolæmia in disease, that in health the range of plasma cholesterol values is considerable. The following figures are the result of examination of about fifty normal individuals of both sexes:—

Grammes of Cholesterol per 100 *Grammes of Plasma.*

	Total Cholesterol.		Free Cholesterol.		Ester Cholesterol.	
	Average.	Limits of Variation.	Average.	Limits of Variation.	Average.	Limits of Variation.
Males . .	0·170	0·11-0·22	0·050	0·005-0·082	0·12	0·08 -0·14
Females . .	0·153	0·08-0·23	0·054	0·02 -0·094	0·10	0·054-0·14

It will be seen at a glance how wide is the range of normal values in different individuals, but in actual fact the level of plasma cholesterol in a single individual is much more constant, and the writer has records of his own plasma cholesterol, taken fasting, over several years, which show but little variation.

The ratio of ester cholesterol to the total cholesterol in the plasma is, in the majority of cases, between 60 and 70 per cent. It should be noted that the cholesterol and cholesterol esters in plasma are not simply present in colloidal solution or suspension as such, but, as the writer and Gainsborough have clearly shown, are in intimate physical or perhaps loose chemical union with the proteins, particularly the globulins.

(2) In the past decade the purely chemical investigation of cholesterol, due largely to the labours of A. Windaus and H. Wieland and their co-workers, has gone far ahead of the physiological or medical side.

Cholesterol, an unsaturated secondary alcohol of the formula $C_{27}H_{46}O$, is derived from the parent saturated hydrocarbon, cholestane $C_{27}H_{48}$, whose molecule consists of four conjugated

https://doi.org/10.1017/S0022215100037427 Published online by Cambridge University Press

hydro-aromatic rings (two six-carbon rings and two five-carbon rings) and an octyl side chain.

Various artificial isomers of cholesterol have been prepared in the laboratory, perhaps the most interesting being the very labile *allocholesterol*, in which the position of the double linkage is changed. It is very readily changed back into ordinary cholesterol. This allocholesterol is very easily reducible in vitro to coprosterol. In adult man the waste cholesterol of the body is excreted mainly through the intestine, for the most part as a mixture of isomeric alcohols, $C_{27}H_{48}O$, viz., bihydrocholesterol and coprosterol, the latter in preponderating quantity. Bihydrocholesterol is the natural reduction product of cholesterol, produced by saturating the double link with hydrogen. In the formation of the isomeric coprosterol a steric rearrangement of the substituent groups at a particular carbon atom has occurred in addition, and the parent hydrocarbon is coprostane, a stereo-isomer of cholestane. The formation of coprosterol is generally believed to occur in the gut and to be due to reduction of cholesterol by intestinal bacteria, but the evidence for this is not too convincing.

The specific bile acids and cholesterol possess the same carbon structure, except that in the bile acids an isopropyl group $(CH_3)_2CH$ has been eliminated from the side chain and the carbon atom, to which it was attached, oxidised to a COOH group.

It is clear from this work that the sterols and bile acids form a group of substances of analogous structure, which is entirely different from that of the usually considered metabolic groups such as proteins, carbohydrates or fats. The bile acids that have been most thoroughly studied are those of ox-bile, viz., lithocholic acid $C_{24}H_{40}O_3$, deoxycholic acid $C_{24}H_{40}O_4$ and cholic acid $C_{24}H_{40}O_5$, which are hydroxyl derivatives of the parent acid, cholanic acid $C_{24}H_{40}O_2$. In goose, ox and human bile another deoxycholic acid, with the two hydroxyl groups in another position, is also found, viz., cheno- or anthropodeoxycholic acid. These acids, though they occur as such in bile, are usually coupled with glycine or taurine. They belong to the same stereoisomeric series as coprosterol, whose parent hydrocarbon is coprostane. However, from pigs' bile a hyodeoxycholic acid has been isolated, and it is the only naturally-occurring acid, so far found, which belongs to the

https://doi.org/10.1017/S0022215100037427 Published online by Cambridge University Press

same stereochemical series as cholesterol. The following scheme shows their relationship:—

Parent hydrocarbon	Cholestane	Coprostane
	↓	↓
Unsaturated alcohol	Cholesterol ⇄	Allocholesterol
	↓	↓
Saturated alcohol	Bihydrocholesterol	Coprosterol
	↓	↓
Bile acids	Hyodeoxycholic acid	Various derivatives of cholanic acid

It is important to note that in vertebrate animals the only sterol isolated, with the exception of small amounts of its reduction product bihydrocholesterol and traces of ergosterol or an alcohol very like it, is common cholesterol; whereas many different bile acids have been isolated from different species of animals. Furthermore, in the different animals the acids, even though the same, are present in very different relative proportions.

On account of the close chemical relationship between bile acids and cholesterol, it has been suggested by Windaus that the bile acids are formed by direct breakdown of cholesterol in the organism. Enderlen, Thannhaüser and Jenke found that intravenous injection of cholesterol in colloidal solution produced no increase in the secretion of bile acid, whereas similar injections of allocholesterol led to noticeable increases of this secretion. The result is significant, but unfortunately the available methods of estimating bile acids are indirect and unsatisfactory, and afford no information as to the relative distribution of the different acids. On the other hand, since Wieland succeeded in synthesising coprostane from cholanic acid, the reverse change appears possible. In the writer's opinion, however, a close consideration of the formulae assigned to these substances suggests that both cholesterol and bile acids are synthesised from some common origin by collateral processes.

(3) In 1916 H. Wieland and S. Sorge enunciated the "choleic acid principle"; they found that deoxycholic acid formed stable molecular compounds with the higher fatty acids, *e.g.*, $8C_{24}H_{40}O_4+C_{18}H_{36}O_2$ with stearic acid. The choleic acids isolated from bile were a mixture of such stearic, palmitic and oleic acid compounds. These compounds go unchanged into the salt condition and are very stable. They crystallise well and their sodium salts are *very easily soluble in water.*

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Besides fatty acids, these observers found that many other substances form somewhat similar compounds with deoxycholic acid, though often more easily dissociable. Materials which are insoluble or nearly so in water, such as naphthaline, xylene, strychnine, quinine, cholesterol, camphor, fat, etc., are brought into solution by an aqueous solution of sodium deoxycholate. This power of uniting with insoluble substances to form soluble products Wieland called the "choleic acid principle" and suggested that it had an important function in transporting insoluble substances, such as fat and fatty acids, etc., through the intestinal wall.

It is perhaps important, in connection with cholelithiasis to note that anthropodeoxycholic acid, isolated from human corpse bile, in which it accompanies ordinary deoxycholic acid, *does not obey* the "choleic acid principle."

This choleic acid principle appears to provide a more satisfactory explanation of intestinal fat absorption than either the theory of particulate absorption or that of the absorption of fatty acids in the form of soluble soaps.

(4) Ox bile has long been known to have a cholagogue activity when given by the mouth. Recent investigations have shown that deoxycholic acid possesses marked cholagogue activity in animals and man, and also that the corresponding keto acid, which can be administered intravenously, is even more potent.

(5) That cholesterol is synthesised in the organism has been clearly proved by balance experiments by Gardner and Fox, and by Thannhaüser, and in other ways by Beumer and Lehmann, H. G. Channon and others.

(6) Cholesterol ingested with the food is absorbed by the alimentary canal, but in this respect the metabolism of herbivora appears to differ very markedly, in degree if not actually in type, from that in carnivora or omnivora.

In the herbivora—ox, sheep, horse, rabbit and hippopotamus—Gardner and other workers have clearly shown that cholesterol is never excreted in the fæces of the healthy animals; and the writer was led to the conclusion that, in the metabolism of these animals, cholesterol is strictly conserved. Evidence was brought forward to show that the cholesterol excreted in the bile, including such as arises from the breakdown of erythrocytes, is completely reabsorbed—the so-called "cholesterol cycle." In such animals any waste of cholesterol must be made up by

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synthesis. Before this possibility was proved many attempts were made by Gardner and others to ascertain whether the vegetable sterols of plants could be absorbed and converted into cholesterol, but the results were inconclusive, owing to the lack of information then as to the variations of cholesterol in normal animal blood. It was shown, however, that the so-called chortosterol of grass was excreted unchanged and practically quantitatively. This chortosterol is, however, possibly not a sterol, but a paraffin alcohol. In recent times Schönheimer and co-workers have been able to demonstrate that plant sterols, ingested in known quantities in the food, are quantitatively excreted, and in tissues behave as foreign bodies, but on the other hand cholesterol, so administered, is very rapidly absorbed. In herbivora the administration of cholesterol, which is not a normal constituent of their food, leads to toxic effects. Prolonged feeding of this substance to rabbits, as is well known, leads to cholesterol infiltration of the various tissues, the changes in which have been considered to resemble human pathological processes, *e.g.*, athero-sclerosis.

In carnivora and omnivora an excretory mechanism appears to exist, so that a quantity of sterol is always excreted in the fæces. This may be cholesterol or coprosterol or both, together with a small amount of bihydrocholesterol. In adult man the bulk of the excreted sterol—80 per cent. or more—consists of coprosterol. The rest is made up of bihydrocholesterol, which appears to be excreted through the intestinal walls, and cholesterol. The quantity of sterol excreted is always greater in quantity by almost 0·3 gram per day than the cholesterol ingested with food. In infants in the milk stage, however, only cholesterol is excreted, and there is only a very slight excess of output over intake, almost within the errors of experiment. At what stage and under what conditions this gives place to coprosterol is as yet unknown.

This change of cholesterol to coprosterol, as was suggested several years ago by the writer, may be one of the factors which limit the absorption of cholesterol. In this connection it is significant that, as Schönheimer has shown, coprosterol administered with food is not absorbed, and in tissue behaves as a foreign body.

However, in carnivora there is no doubt that cholesterol is absorbed from the food and reaches the blood-stream in partially esterified form through the lymph, though some may enter the

https://doi.org/10.1017/S0022215100037427 Published online by Cambridge University Press

portal circulation directly. In carnivora and omnivora no one has ever produced by high cholesterol feeding such pathological phenomena as occur in rabbits.

(7) In man, as in other animals, *e.g.* cats and rabbits, the level of the cholesterol content of the plasma, taken fasting (*i.e.*, before breakfast), can be raised or lowered by *sufficiently* prolonged feeding with diets of high or low cholesterol content. Such changes are most marked as regards the cholesterol in ester form. As a result of a single meal, alimentary hypercholesterolæmia in the ordinarily accepted sense does not occur and there is no obvious connection between the amount of cholesterol ingested and the cholesterol level of the plasma during digestion. During the digestive process, changes in the cholesterol content of plasma frequently occur, sometimes in a positive and sometimes in a negative direction compared with fasting values. Often a temporary disturbance of the ester to total ratio is observed. These peculiar changes cannot be explained by the influx of cholesterol absorbed from the alimentary canal, but are evidence of an active endogenous metabolism, in which cholesterol takes part, during digestion.

Apart from the changes just mentioned, the only physiological condition in which the value of the plasma cholesterol is altered markedly is in pregnancy. In this condition, from about the twentieth week, the free cholesterol increases to a maximum, which it reaches about the thirtieth week, and concurrently the ester cholesterol falls to a minimum. From this time the amount of free cholesterol decreases and the ester increases till an almost normal ratio of free and ester cholesterol is reached before or near parturition. The plasma cholesterol values usually become quite normal in the first or second week of the puerperium.

This characteristic picture has, so far, not been explained.

Cholesterol level in certain pathological conditions.—In numerous papers in medical literature hypo- or hypercholesterolæmia have been stated to occur in a large variety of diseased conditions, but in very many of the cases the changes noted have been really within the limits of normal variations.

Hypocholesterolæmia certainly does occur, but at the height of the fever only, in a variety of febrile and infective conditions, but the meaning of this is not yet understood.

True hypercholesterolæmia occurs in certain types of nephritis: hypothyroidism, biliary stasis and diabetes.

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Nephritis.—Hypercholesterolæmia is present in all those cases which possess the "nephrotic" syndrome, marked by diffuse parenchymatous degeneration, heavy albuminuria, changes in the plasma proteins, and where œdema is the chief symptom. Consequently increases of plasma cholesterol are found in these cases of acute nephritis of prolonged duration, when albuminuria is persistent and hæmaturia absent, and, as Gainsborough has pointed out, in all that group of chronic nephritis which includes, at one end of the scale, the so-called pure "nephrosis," and at the other end, cases of parenchymatous nephritis, in which the initial "nephrosis" is more or less rapidly followed by sclerosis and contraction of the kidneys. The total cholesterol in the plasma may reach as much as 1 per cent., and the percentage of total in form of ester may assume abnormal value in either direction. This high level of cholesterol is present throughout the course of these cases of nephritis, so long as there is any tendency to œdema, and it persists in cases of subacute parenchymatous nephritis for long periods, even though œdema is temporarily absent. After a more or less prolonged period the tendency to œdema is replaced by tendency to vascular changes, increase of blood-pressure and signs of nitrogen retention, and about the same time the hypercholesterolæmia diminishes.

Hypercholesterolæmia is not found in cases of interstitial nephritis or in arteriosclerotic kidney or in arteriosclerosis.

The cause of this hypercholesterolæmia is not known, but may perhaps be related to the disturbance of the plasma proteins resulting from proteinuria.

Myxœdema.—The total cholesterol of the plasma in this condition may be as much as 50 per cent. above normal, but after administration of thyroid gland the value generally approaches normal.

Biliary stasis.—Hypercholesterolæmia has been stated to occur in cholelithiasis and in affections of the liver, but there can be little doubt that in all the earlier investigations the hypercholesterolæmia noted was due to the biliary stasis and not to the pathological condition prior to this. Hypercholesterolæmia is not a feature of uncomplicated cholelithiasis. If, however, biliary stasis occurs, *e.g.*, from impaction of a stone in the common bile duct, or from catarrhal jaundice, the blood-picture shows very interesting changes. In the early stages the cholesterol ester rapidly diminishes and may almost disappear

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from the plasma, while the free cholesterol remains normal or shows a slight increase. This characteristic change of the plasma cholesterol may also be observed in those cases of cholecystostomy in which none of the bile is reaching the intestines.

This change must be due to the disturbances of fat and cholesterol absorption from the intestines in consequence of the absence of bile.

If complete biliary obstruction persists for many weeks, then the plasma cholesterol gradually increases, both in free and ester forms, until marked hypercholesterolæmia occurs, but nevertheless the percentage of total cholesterol as ester remains low and is usually from 30 to 40 per cent.

If the biliary obstruction is not quite complete or is intermittent, the marked hypercholesterolæmia may not appear.

Diabetes.—True hypercholesterolæmia sometimes occurs in severe cases of diabetes.

Hypercholesterolæmia in diabetes and also in biliary obstruction may lead to deposition of cholesterol in the skin as xanthoma.

Skin.—Our knowledge of the biochemistry of skin is very defective, but there can be little doubt that it is one of the minor paths for cholesterol excretion. Cholesterol, cholesterol esters and bihydrocholesterol have been isolated from skin and skin secretions. Earlier investigators stated that in human skin no lanoline was present—mainly on histological grounds. Lanoline, the fat of sheep's wool, has been subjected to much investigation. In addition to cholesterol and cholesterol ester and various fats, it contains a substance called isocholesterol, which was supposed to be an isomer of cholesterol, and was also said to occur in the latex of certain rubber plants. It belongs to the group of sterols which do not form insoluble compounds with digitonin. Recently Windaus has shown that this isocholesterol is not a single substance, but consists of two sterols, crystallising together like a single substance, which he names agnosterol and lanesterol. Agnosterol contains three double linkages and lanesterol two; and moreover their molecules contain thirty carbon atoms and not twenty-seven, so that they are not simply related to cholesterol.

Clearly, skin will repay closer investigation, though the subject is beset with difficulties.

https://doi.org/10.1017/S0022215100037427 Published online by Cambridge University Press

Cholesterol Metabolism

Während der vergangenen 20 Jahre wurden zahlreiche Arbeiten über den Cholesteringehalt im Blute von Kranken veröffentlicht. Der Verfasser lenkt die Aufmerksamkeit auf gewisse biochemische Tatsachen, die in den letzten Jahren gesammelt wurden. Er zeigt, dass selbst beim Gesunden der Gehalt an Plasma- und Cholesterinwerten bedeutend ist. Beim Menschen kann der Cholesteringehalt des Plasmas erhöht oder vermindert werden durch genügend lange Ernährung mit einer cholesterin-reichen oder -armen Diät. Unter- oder Ueber-Cholesterinaemie wurden bei einer grossen Zahl von krankhaften Zuständen gefunden; oft finden sie sich auch innerhalb der normalen Variationsbreite.

Au cours des 20 dernières années, il y a eu de nombreuses publications sur le dosage de la cholestérinémie au cours des maladies. L'auteur attire l'attention sur certains faits bio-chimiques qui se sont accumulés au cours des dernières années. Il montre que les variétés de la teneur du plasma en cholestérine, même a l'état normal, est considérable. Chez l'homme, la teneur du plasma en cholestérine peut être augmentée ou diminuée par un régime sufisament prolongé et plus ou moins riche en cholestérine. L'hypo et l'hyper cholestérinémie sont considerées comme survenant dans un grand nombre de maladies, mais souvent ces variations se maintiennent dans la limite des variations normales. M. V.

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